

Distinct Molecular Pathways Driving Thymic and Pulmonary Neuroendocrine Neoplasms

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Abstract

Thymic and pulmonary neuroendocrine neoplasms (TNEN and PNEN) are morphologically similar but arise from distinct embryologic tissues. Whether these similarities extend to shared molecular programs is unclear. We performed an integrative multi-omics comparison of formalin-fixed paraffin-embedded samples from 45 TNEN and 38 PNEN using immunohistochemistry, next-generation sequencing, NanoString RNA profiling, and mass spectrometry-based proteomics. Transcriptomic and proteomic data were jointly modeled using MuVI, a Bayesian multi-view latent factor model incorporating Hallmark gene sets. CD44 expression was validated in 403 thymic, pulmonary, and gastrointestinal and pancreatic NEN. Integrative analysis revealed four molecularly distinct subtypes along two axes—differentiation grade and organ of origin. Neuroendocrine carcinomas (NEC) from both organs shared cell cycle activation (E2F targets, G2/M checkpoint, Myc targets) with metabolic depletion and frequent TP53/RB1 co-inactivation but diverged in immune profiles: pulmonary NEC displayed an immune-hot phenotype, whereas thymic NEC showed predominant epithelial–mesenchymal transition activity. Pulmonary NEC harbored substantially higher tumor mutational burden than thymic NEC. Neuroendocrine tumors (NET) from both organs exhibited proliferative quiescence but diverged metabolically: pulmonary NET showed broad metabolic activation and stress signaling, while thymic NET were transcriptionally quiescent with PI3K/AKT/mTOR as the sole significantly enriched pathway (FDR < 0.05). Thymic NET G3 clustered with lower-grade TNET rather than NEC. CD44 emerged as a novel immunohistochemical marker of pulmonary NET. These findings demonstrate that thoracic NEN segregate into molecular entities driven by anatomical origin and tumor grade, with divergent immune phenotypes and PI3K/AKT/mTOR dependence in thymic NET as potential targets for site-adapted therapeutic strategies.

Introduction

Neuroendocrine neoplasms (NEN) of the thymus and lungs are morphologically similar and classified using the same WHO criteria [1] (Fig. 1). The revised classification has adopted elements of the common classification framework for NEN [2], separating these tumors into neuroendocrine tumors (NET G1, G2, G3) and neuroendocrine carcinomas (NEC), comprising large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCC). In this article, framework terminology (NET G1/G2 instead of typical/atypical carcinoid) will be used when referring to carcinoid tumors of the lung (PNET) and thymus (TNET).

Published molecular data from both lung and thymus have been shown to categorize thoracic NEN into three primary clusters, which are enriched for NET G1, NET G2, and NEC, respectively [3, 4]. Furthermore, in both the thymus and the lung, it has been demonstrated that the spectrum of NETs comprises a distinct additional subtype of tumors with intermediate malignancy that has been designated as NET G3 [4] in the thymus, or “atypical carcinoid with elevated mitotic counts and/or Ki67 index” in the lung [5, 6]. The distinguishing features of this subtype include carcinoid morphology, lower genomic instability [6], a lower proliferation rate, preservation of chromogranin expression, and low EZH2 expression [4]. These and other observations, including the ESMO LungNENomics study by Mathian et al. [7], have shown that

purely morphology-based grading fails to capture the full molecular diversity of thoracic NEN and highlights the necessity of an integrated morpho-molecular framework.

It is currently unknown whether the morphological similarities between thymic and pulmonary NEN extend to molecular features and tumor cell biology. However, the thymus and lungs are derived from different embryonic structures: the thymus arises from the endoderm of the third pharyngeal pouch, which also generates the parathyroid glands [8], whereas the lungs represent outpouchings of the ventral wall of the primitive foregut [9]. Consequently, it is plausible that tumors in these organs may evolve along diverse molecular pathways. In support of this prediction, there are striking epidemiological differences. In the thymus, 50% of cases are TNET G2, followed by TNET G1 (20%), with a marked male predominance. In contrast, in the lung, SCC accounts for roughly 80% of all NEN, while PNET G2 are distinctly rare. Furthermore, smoking has not been identified as a known risk factor for thymic NEC.

In contrast to PNEN, the mutational landscape of TNEN remains poorly characterized. PNET show recurrent mutations of *MEN1*, *ARID1A*, *EIF1AX*, *ATM*, *PSIP1*, *ROBO1*, and subunits of the SWI/SNF complex [3, 10]. In the present study, we systematically compared a series of thymic and pulmonary NEN using proteomic and gene expression analyses, as well as comprehensive next-generation sequencing, to further characterize organ-specific similarities and differences in the biology of these tumors.

Materials and Methods

For this study, we used archival formalin-fixed, paraffin-embedded (FFPE) tissue slides or blocks of n = 45 TNEN and n = 38 PNEN (see Table 1) classified according to the current WHO system [1]. Large parts of the TNEN series had been characterized previously [4, 11] and contained n = 4 cases classified as TNET G3. To determine CD44 expression across NEN from different organs, we used n = 211 gastrointestinal, n = 174 pulmonary, and n = 18 thymic NEN organized on multi-tissue arrays. The study was approved by the University Medical Center Göttingen ethics committee (no. Dok_7_2016) and conducted in accordance with the Declaration of Helsinki.

Table 1
Patient and tumor sample characteristics

Characteristics	TNEN (n = 45)	PNEN (n = 38)
median age (years, range)	60.5 (25–100)	63.5 (30–79)
Age < 60 years (n)	20	12
Age ≥ 60 years (n)	25	26
Gender		
Female (n)	8	21
Male (n)	37	17
Grading (n)		
NET G1	8	7
NET G2	22	20
NET G3	7	0
LCNEC	5	9
SCC	3	2

Immunohistochemistry

Immunohistochemistry (IHC) was performed on a Dako Autostainer platform using Dako EnV FLEX Peroxidase detection. CD44 antibody (Sigma-Aldrich, 1:1000) was evaluated using a three-tiered scoring system for staining intensity (details in **Online Resource 1**).

Mass spectrometry and proteomic analysis

FFPE tissue samples were processed for mass spectrometry as previously reported [12]. Briefly, tumor regions containing ≥ 80% tumor cells were macrodissected and mixed 1:1 with a Super-SILAC protein standard composed of four neuroendocrine cell lines (UMC11, H727, BON1, NET18P). Samples were processed by filter-aided sample preparation (FASP) and analyzed by nano-HPLC coupled to an Orbitrap Fusion mass spectrometer. MS/MS spectra were analyzed in MaxQuant against the UniProtKB/Swiss-Prot human reference proteome with SILAC-based quantification. Detailed protocols are provided in **Online Resource 1**.

Gene expression profiling

RNA was isolated from FFPE sections using the RNeasy FFPE Kit (Qiagen). 200 ng RNA per sample was hybridized and analyzed on the nCounter Analysis System (NanoString) using the PanCancer Pathway

Panel covering 770 genes of 13 cancer-associated canonical pathways. Quality control and normalization details are provided in **Online Resource 1**.

Next-generation sequencing

Targeted sequencing was performed using the TruSight Oncology 500 (TSO500) panel (Illumina) [13], covering 523 cancer-related genes for detection of single- and multiple-nucleotide variants, copy-number variants, microsatellite instability, and tumor mutational burden (TMB). For a subset of cases, whole-exome sequencing (WES, $n = 5$) and OncoPrint Comprehensive Panel v3 ($n = 1$) were performed. TMB values are reported for the TSO500 subcohort only. Sequencing details are provided in **Online Resource 1**.

Bioinformatic and statistical analyses

Transcriptomic and proteomic data were subjected to systematic preprocessing and quality control (**Online Resource 1**). NanoString data were normalized using a two-step procedure: positive control normalization followed by housekeeping gene normalization. After removal of control probes, normalized counts were converted to counts per million (CPM) and log₂-transformed with a pseudocount of one. For proteomics, only proteins quantified in at least 50% of samples were retained. Dimensionality reduction was performed using principal component analysis (PCA) and t-distributed stochastic neighbor embedding (t-SNE). Differential expression analyses were performed by comparing predefined tumor subgroups using Welch's two-sample t-test with Benjamini-Hochberg FDR correction. Effect sizes were estimated using Cohen's d .

For integrative analysis of transcriptomic and proteomic data, we applied MuVI, a Bayesian multi-view latent factor model that enables joint representation learning across molecular layers while incorporating prior biological knowledge via structured sparsity. RNA and proteomics data were modeled as two Gaussian views and jointly embedded into a shared latent space comprising 32 latent factors. Hallmark gene sets from the Molecular Signatures Database (MSigDB) were ingested as prior information, yielding 31 informed latent factors complemented by one uninformed factor to capture residual variation. Latent factor scores were subsequently used to assess pathway-level activity differences between predefined tumor subgroups.

Results

Distinct molecular programs in pulmonary and thymic neuroendocrine neoplasms

We compared 11 PNEC ($n = 2$ SCC, $n = 9$ LCNEC) and 26 PNET ($n = 6$ NET G1, $n = 20$ NET G2) with 7 TNEC ($n = 2$ SCC, $n = 5$ LCNEC) and 31 TNET ($n = 7$ NET G1, $n = 20$ NET G2, $n = 4$ NET G3) using RNA expression profiling and proteomics.

Both RNA and protein profiles revealed distinct clusters by grade and tissue origin (Fig. 2a + b). PNEC and TNEC were indistinguishable based on highly similar profiles, supporting shared molecular

mechanisms in NEC across both organs. In contrast, PNET and TNET exhibited characteristic separate molecular profiles. TNET G1/G2 and G3 showed significant overlap, indicating a continuous molecular transition.

At the RNA level, the most significant genes distinguishing TNET (G1-G3) from PNET (G1/G2) included CD44, BAMBI, WNT4, CCND2, CDK6, CPA3, SERPINA1, MMP7, MAP3K5 and EGFR (higher in lung), as well as NECTIN1, IL34, JAG2, MAPK10 and SOCS1 (higher in thymus) (Fig. 2c). Among the most significant differentiating proteins were CD44, CAPN2, HSPB1, SCGN, DDC, QDPR, IDH1, ALDH1A1, PCK1 and MAOA (higher in lung), whereas PFKFB2 was higher in thymus (Fig. 2d). Thus, PNET showed increased activity of pathways related to WNT signaling and cell cycle regulation (e.g. WNT4, BAMBI, CCND2, CDK6), as well as cell adhesion and extracellular matrix remodeling (CD44, MMP7), accompanied by markers of secretory and stress response (SERPINA1, CPA3, SCGN, HSPB1). In contrast, TNET showed higher expression of genes involved in cell adhesion and junctional signaling (NECTIN1), immune and cytokine modulation (IL34, JAG2), and MAPK-related signaling (MAPK10, SOCS1). Since CD44 emerged as a distinguishing feature between PNET and TNET both at the RNA and the protein level (Fig. 3a), we confirmed its expression in gastrointestinalpancreatic (n = 211), pulmonary (n = 174), and thymic (n = 18) NEN by immunohistochemistry. High expression of CD44 was significantly more frequently observed in NET than in NEC in all organs and was highest in PNET (Fig. 3b).

In a next step, we analyzed the most significant gene transcripts and proteins responsible for the difference between pooled pulmonary and thymic NEC vs. NET (Fig. 2e + f). Both datasets demonstrated a marked shift from differentiation- and metabolism-associated programs in NET toward cell-cycle, replication, and DNA repair dominance in NEC. NET showed relative enrichment of proteins and transcripts associated with metabolic regulation, vesicular transport, and cellular differentiation, including PURA, ALDH5A1, PFKFB2, MAPRE3, SCG5, PCSK1N, and WNT4, consistent with a more differentiated, metabolically active phenotype. In contrast, NEC showed pronounced upregulation of gene transcripts involved in cell-cycle control and genomic maintenance, including UBE2C, CDC20, CENPF, RRM2, KIF2C, MELK, MKI67, and EZH2 (Fig. 2e). At the proteomic level, NEC showed increased expression of replication-associated proteins such as MCM2–MCM7 and KPNA2, supporting tight coupling of DNA synthesis, nuclear transport, and chromatin assembly [14] (Fig. 2f). Both pulmonary and thymic NEC shared this transcriptional and proteomic proliferation signature, although the signal was most pronounced in PNEC.

The tissue of origin determines the mutational burden in thoracic neuroendocrine tumors

Next-generation sequencing using the TSO500 panel was performed on n = 20 pulmonary (n = 2 SCLC, n = 8 LCNEC, n = 3 NET G1, n = 7 NET G2) and n = 20 thymic NEN (n = 2 SCC, n = 5 LCNEC, n = 3 NET G1, n = 7 NET G2, n = 5 NET G3). In addition, n = 5 cases were analyzed by whole exome sequencing (n = 1 TNET G1, n = 1 TNET G2, n = 2 TNET G3, n = 1 thymic SCC), and n = 1 TNET G3 was analyzed using the Oncomine Comprehensive Panel v3. In total, 228 variants were identified in PNEC, 142 in PNET, 61 in TNEC, 139 in TNET G1/G2, and 44 in TNET G3.

Each variant was classified according to established clinical significance criteria. Across all subgroups, benign and likely benign variants predominated. A chi-square test of independence revealed a statistically significant difference ($\chi^2=18.69$, $df = 2$, $p = 8.7 \times 10^{-5}$) in the distribution of variant classifications between NEC (PNEC + TNEC) and NET (PNET + TNET G1-G3). High-grade tumors exhibited a significantly higher proportion of pathogenic variants, whereas low-grade tumors were dominated by benign or likely benign variants. PNEC exhibited substantially higher TMB (mean 12.1, range 8.9–16.8) compared to TNEC (mean 3.9, range 0–8.7), and the highest proportion of pathogenic and likely pathogenic alterations (57.1%), mainly affecting *TP53* and *RB1*. The tumor burden was only slightly increased in TNEC compared to TNET (TMB 3.9 in TNEC vs. 2.1 in TNET). All NET (PNET and TNET) displayed comparatively few pathogenic variants, including *MEN1*, *MUTYH*, and *PIK3C2G* (Table 2 and **Supplementary Table S1**). PNET and TNET showed largely similar overall variant distributions. A few cases with high-level gene amplifications ($n = 1$ LCNEC with *FGFR1*, $n = 1$ SCLC with *FGFR1* and *MYCL*) or gene fusions (one LCNEC with *KIT::CLOCK* fusion, one *ADAM9::FGFR1* fusion) were all lung tumors (Fig. 4). There were no cases (0/40) with microsatellite instability.

Table 2
Pathogenic variants across neuroendocrine tumor subgroups

Gene	PNEC (n = 9)	PNET (n = 10)	TNEC (n = 5)	TNET G1/G2 (n = 14)	TNET G3 (n = 7)	Total (n = 45)
TMB, mean (range)	11.7 (6.5–16.8)	2.2 (0.8–4.0)	3.3 (0–8.7)	2.0 (0–4.4)	2.1 (0–4.2)	—
Recurrently mutated genes (≥ 2 cases)						
TP53	8	0	4	1	0	13
RB1	4	0	2	0	0	6
MEN1	0	1	0	3	1	5
MUTYH	0	0	0	2	1	3
FGFR1	2	0	0	0	0	2
PIK3C2G	2	0	0	0	0	2
STK11	2	0	0	0	0	2
HRAS	0	0	0	1	1	2
MYCL	1	0	1	0	0	2
FAM98B	0	0	1	1	0	2
Selected singletons (clinically relevant)						
ARID1A	0	1	0	0	0	1
DAXX	1	0	0	0	0	1
NOTCH3	0	0	1	0	0	1
PTEN	0	0	1	0	0	1
NRAS	0	0	0	1	0	1
EIF2AK4	0	0	0	1	0	1
Structural variants / fusions						
KIT-CLOCK fusion	1	0	0	0	0	1
Complete variant data for all 45 sequenced cases are provided in Supplementary Table S1. TMB: tumor mutational burden (mut/Mb, mean and range; TSO500 subcohort). PNEC: pulmonary NEC (SCC+LCNEC); PNET: pulmonary NET G1/G2; TNEC: thymic NEC; TNET G1/G2: thymic NET G1/G2; TNET G3: thymic NET G3. Values: cases with ≥ 1 pathogenic/likely pathogenic variant. 0 (—): no pathogenic variant detected. Fusions: Archer Pan Solid Tumor panel.						

Gene	PNEC (n = 9)	PNET (n = 10)	TNEC (n = 5)	TNET G1/G2 (n = 14)	TNET G3 (n = 7)	Total (n = 45)
ADAM9-FGFR1 fusion	1	0	0	0	0	1
<p><i>Complete variant data for all 45 sequenced cases are provided in Supplementary Table S1. TMB: tumor mutational burden (mut/Mb, mean and range; TSO500 subcohort). PNEC: pulmonary NEC (SCC+LCNEC); PNET: pulmonary NET G1/G2; TNEC: thymic NEC; TNET G1/G2: thymic NET G1/G2; TNET G3: thymic NET G3. Values: cases with ≥ 1 pathogenic/likely pathogenic variant. 0 (–): no pathogenic variant detected. Fusions: Archer Pan Solid Tumor panel.</i></p>						

Integrative pathway analysis reveals distinct molecular programs across neuroendocrine tumor subtypes

Integrative multi-omics analysis using MuVI delineated four transcriptionally and proteomically distinct NEN phenotypes (Fig. 5).

Neuroendocrine carcinomas share proliferative activation and metabolic depletion

Both NEC subtypes—PNEC and TNEC—converged on a shared molecular signature characterized by activation of canonical cell cycle programs (E2F targets, G2/M checkpoint, Myc targets V1, DNA repair) and concurrent depletion of central metabolic pathways, including oxidative phosphorylation (PNEC: – 0.80; TNEC: – 0.74, FDR < 0.05), glycolysis (PNEC: – 0.76; TNEC: – 0.96, FDR < 0.05), and fatty acid metabolism. This combination of high proliferative transcriptional output with suppressed metabolic pathway activity is consistent with a poorly differentiated phenotype in which biosynthetic resources are channeled toward cell division rather than specialized metabolic function. In line with their mutational signatures (Fig. 4), both NEC subtypes exhibited depletion of p53 pathway activity (PNEC: – 0.94; TNEC: – 0.43). Despite this shared proliferative-metabolic architecture, PNEC and TNEC diverged substantially in their immune and invasive profiles. PNEC displayed the strongest immune activation of all four clusters, with pronounced enrichment of allograft rejection (1.38), inflammatory response (1.28), interferon- γ signaling (1.16), and complement (1.12), consistent with an immune-hot tumor microenvironment. In contrast, TNEC showed only moderate immune pathway activity but was distinguished by the highest epithelial–mesenchymal transition (EMT) activity across the entire cohort (~ 0.99), accompanied by enrichment of protein secretion and heme metabolism. This pattern suggests that while PNEC engages in active immune interaction, TNEC employs a distinct biological strategy in which dedifferentiation and mesenchymal reprogramming predominate, a mechanism increasingly recognized as a driver of immune evasion in thoracic malignancies [15].

Discussion

Thymic and pulmonary neuroendocrine neoplasms are morphologically similar and have been classified using the same WHO criteria across all editions of the thoracic tumor classification. However, the

thymus and lung arise from different embryonic structures, and both their relative frequencies and clinical backgrounds differ substantially. The present study is the first to systematically compare the transcriptomic, proteomic, and genomic profiles of a large cohort of TNEN and PNEN across all WHO-defined subtypes including thymic NET G3. To our knowledge, this is also the first published application of mass-spectrometry-based proteomics to thymic neuroendocrine tumors. Our integrated multi-omics data provide three principal findings: (1) PNET and TNET are driven by fundamentally different molecular programs defined by organ of origin; (2) high-grade NEC from both organs converge on shared proliferative and DNA replication signatures but diverge in their immune and invasive profiles; and (3) thymic NET G3 cluster with TNET G1/G2 rather than with NEC, consolidating the concept of a biological continuum of thymic grade 1–3 NET.

RNA expression and protein profiles converged on distinct molecular clusters defined by tumor grade and tissue of origin. PNET and TNET formed separate clusters in both transcriptomic and proteomic analyses, indicating that the molecular divergence between these tumors is maintained across multiple levels of gene regulation. Integrative pathway analysis using MuVI revealed that PNET were characterized by broad metabolic activation—including oxidative phosphorylation, fatty acid metabolism, glycolysis, and hypoxia-adaptive programs—accompanied by active stress and inflammation signaling, while TNET presented a transcriptionally quiescent phenotype with PI3K/AKT/mTOR as the sole significantly enriched pathway (FDR < 0.05). Among the differentially expressed proteins, CD44 was validated in a larger NEN cohort by immunohistochemistry as a novel marker for the distinction between PNET (higher) and TNET (lower).

NEC from both organs clustered together in transcriptomic and proteomic analyses, indicating convergent molecular programs despite different tissues of origin. The shared NEC signature was dominated by activation of cell-cycle and DNA replication pathways, including upregulation of MCM2–7, and was accompanied by a high rate of concurrent *TP53* and *RB1* inactivation (6 of 14 NEC cases, 43%). Upregulation of the minichromosome maintenance complex (MCM2–7) in NEC is a notable finding. MCM2–7 forms the core of the eukaryotic replicative helicase and its abundance in proliferating cells provides a buffer against replication stress [14]. Elevated MCM expression has been reported in neuroendocrine prostate cancers [16], gastroenteropancreatic NEN [17], and neuroblastoma [18], with high levels correlating with poor survival and metastatic potential. Therapeutic targeting of MCM2–7/CMG helicase activity is being explored as a strategy to selectively affect proliferating cancer cells, but clinical translation is pending [19]. EZH2, another top upregulated gene in NEC, has been previously described as an important prognostic factor in pulmonary and thymic NEN that was also instrumental in the immunohistochemical distinction of NET G3 and NEC [4].

Despite their shared proliferative signature, PNEC and TNEC diverged substantially in their immune and invasive profiles. PNEC displayed the strongest immune activation of all four clusters, consistent with an immune-hot tumor microenvironment. In contrast, TNEC showed the highest epithelial–mesenchymal transition (EMT) activity across the entire cohort. EMT has been increasingly recognized as a mechanism of immune evasion in thoracic malignancies: mesenchymal tumor phenotypes are

associated with upregulation of multiple immune checkpoints, enrichment of immunosuppressive regulatory T cells, and downregulation of antigen presentation machinery [15]. The observation that TNEC combines high EMT activity with comparatively lower immune engagement suggests that mesenchymal reprogramming may constitute an alternative immune escape strategy in thymic neuroendocrine carcinomas. Although PNEC and TNEC clustered together at the transcriptomic and proteomic level, their mutational profiles revealed additional differences: PNEC exhibited a substantially higher tumor mutational burden (mean TMB 12.1 mut/Mb) than TNEC (mean 3.9 mut/Mb), consistent with their association with cigarette smoking. The mutational landscape of PNET and TNET was characterized by few pathogenic variants; the most recurrently affected gene was *MEN1*, followed by *MUTYH* and *HRAS*, in good agreement with previous reports in pulmonary NET [10].

The identification of PI3K/AKT/mTOR signaling as the sole significantly enriched pathway in TNET provides a molecular rationale for mTOR inhibitors such as everolimus. While everolimus is approved for gastroenteropancreatic NET [20], evidence for thymic NET remains limited [21]. Our data suggest that TNET may be particularly dependent on this axis. Conversely, the EMT-driven, immune-cold phenotype of TNEC is consistent with data linking mesenchymal reprogramming to poor checkpoint inhibitor response [22].

A central finding of this study is the further molecular characterization of thymic NET G3. In both transcriptomic and proteomic analyses, TNET G3 clustered with TNET G1/G2 rather than with NEC. The mutational burden in TNET G3 was comparable to that of TNET G1/G2 (mean TMB 2.1 mut/Mb in both groups), and TNET G3 lacked the *TP53/RB1* co-mutations characteristic of NEC. *MEN1* mutations were observed in TNET G3 (1/4 cases), further aligning this group with the lower-grade thymic NET. These findings are consistent with the proposal that thymic NET G3 represents a biologically distinct intermediate-grade subtype characterized by increased proliferative activity but retaining the molecular programs of differentiated NET [4]. Our proteomic data—the first available for thymic NET—provide independent multi-omics evidence that TNET G3 is molecularly distinct from NEC and should not be conflated with carcinomas.

Several limitations should be considered. First, this is a retrospective study using FFPE tissue collected from multiple contributing institutions over decades, which may have affected protein and RNA quality. Second, sequencing was performed using three different platforms (TSO500, WES, Oncomine) with differing genomic coverage. However, the key recurrently mutated genes—*TP53*, *RB1*, *MEN1*, *HRAS*, *STK11*—are covered by all platforms, ensuring comparability of the core mutational landscape. TMB values are reported for the TSO500 subcohort only. Finally, the number of TNET G3 cases (n = 7) is small, and larger cohorts will be needed to confirm the findings of this study.

In conclusion, thoracic NEN segregate into molecular entities organized along two axes: differentiation grade and anatomical origin. PI3K/AKT/mTOR signaling and divergent immune phenotypes represent potential targets for site-adapted therapeutic strategies. Thymic NET G3 aligns molecularly with lower-grade TNET, supporting its recognition as a biologically distinct subtype.

Declarations

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Competing Interests

The authors declare no competing interests.

Ethics Approval

This study was approved by the University Medical Center Göttingen ethics committee (no. Dok_7_2016) and conducted in accordance with the Declaration of Helsinki.

Consent to Participate

The ethics committee waived the requirement for informed consent due to the retrospective nature of the study and the use of archival tissue samples.

Data Availability

Transcriptomic profiling data have been deposited in NCBI's Gene Expression Omnibus (GEO) under accession number GSE213609. Targeted DNA sequencing data are available from the European Nucleotide Archive (ENA) under study accession PRJEB56122. The mass spectrometry-based proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with dataset identifier PXD036502.

Author Contributions

HB, AM, and PS designed and executed the study and were responsible for histologic diagnoses. SK, LF, KO, DF, CV, OE, and DM prepared the cases and performed immunohistochemical and RNA-based analyses. AQ and FB were responsible for bioinformatic analyses. HB, JB, CL, HU, and TO performed the proteomic analyses. NN, KRJ, and TR performed the molecular analyses and variant calling. AOK, MJ, TRO, AvH, MH, and SW contributed cases and medical oncological expertise. HB, SK, AM, and PS wrote the manuscript. All authors revised and approved the manuscript.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work the author(s) used Claude AI (Anthropic) to assist with language editing and formatting suggestions. The author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Figures

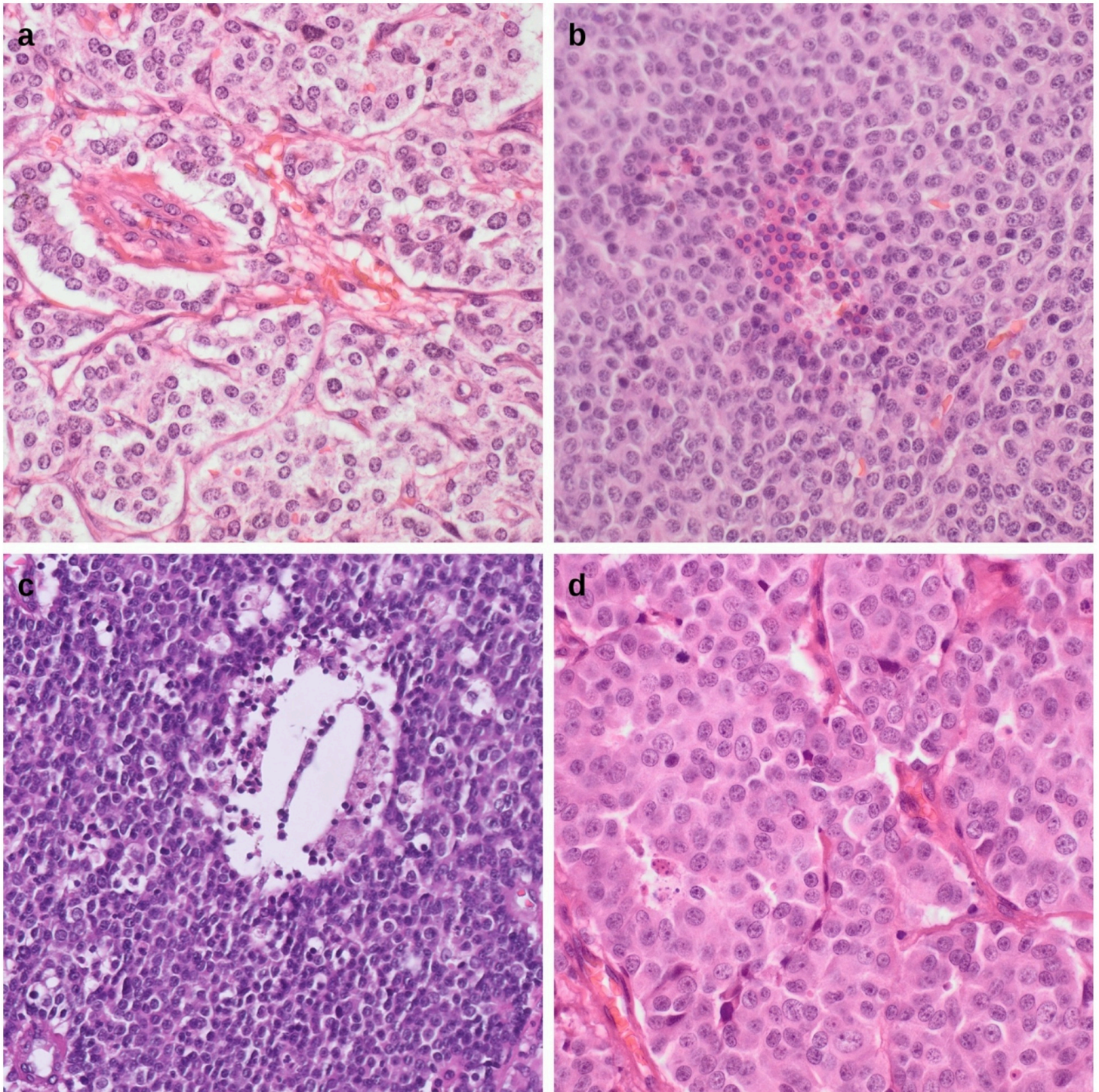


Figure 1

Histopathological features of thymic neuroendocrine neoplasms (H&E stainings). **a** High-power view of thymic neuroendocrine tumor (TNET) G1 (typical carcinoid) showing classic organoid and nested growth patterns with prominent vascularization. **b** TNET G2 (atypical carcinoid) showing a subtle focus of tumor necrosis. **c** TNET G3 with central comedo necrosis. **d** Large cell neuroendocrine carcinoma (LCNEC) with solid sheets of tumor cells exhibiting frequent apoptotic bodies and multiple mitotic figures.

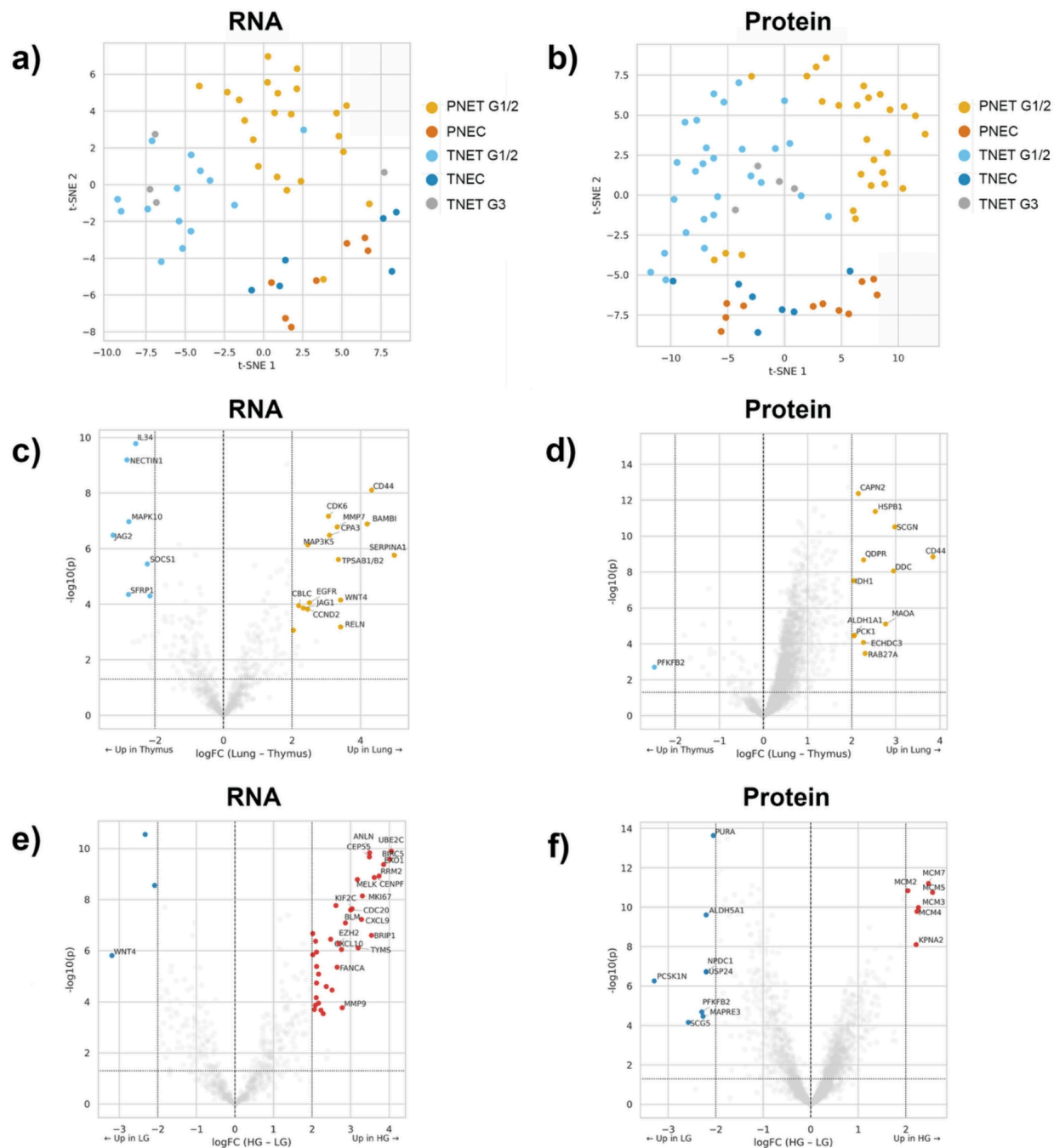


Figure 2

RNA and proteome profiling of pulmonary and thymic neuroendocrine neoplasms. Dimensionality reduction of RNA expression data (**a**; PCA/UMAP) and proteomic data (**b**) demonstrate distinct clustering by tumor grade and tissue of origin. Pulmonary and thymic neuroendocrine tumors (PNET and TNET) form separate clusters, whereas pulmonary and thymic neuroendocrine carcinomas (PNEC and TNEC) cluster together. TNET G3 clustered together with TNET G1/G2, suggesting a molecular

continuum. **c + d)** Volcano plots depicting the most significant gene transcripts (**c**) and proteins (**d**) differentiating PNET and TNET. **e+f)** Volcano plots depicting the most significant gene transcripts (**e**) and proteins (**f**) differentiating NEC vs. NET.

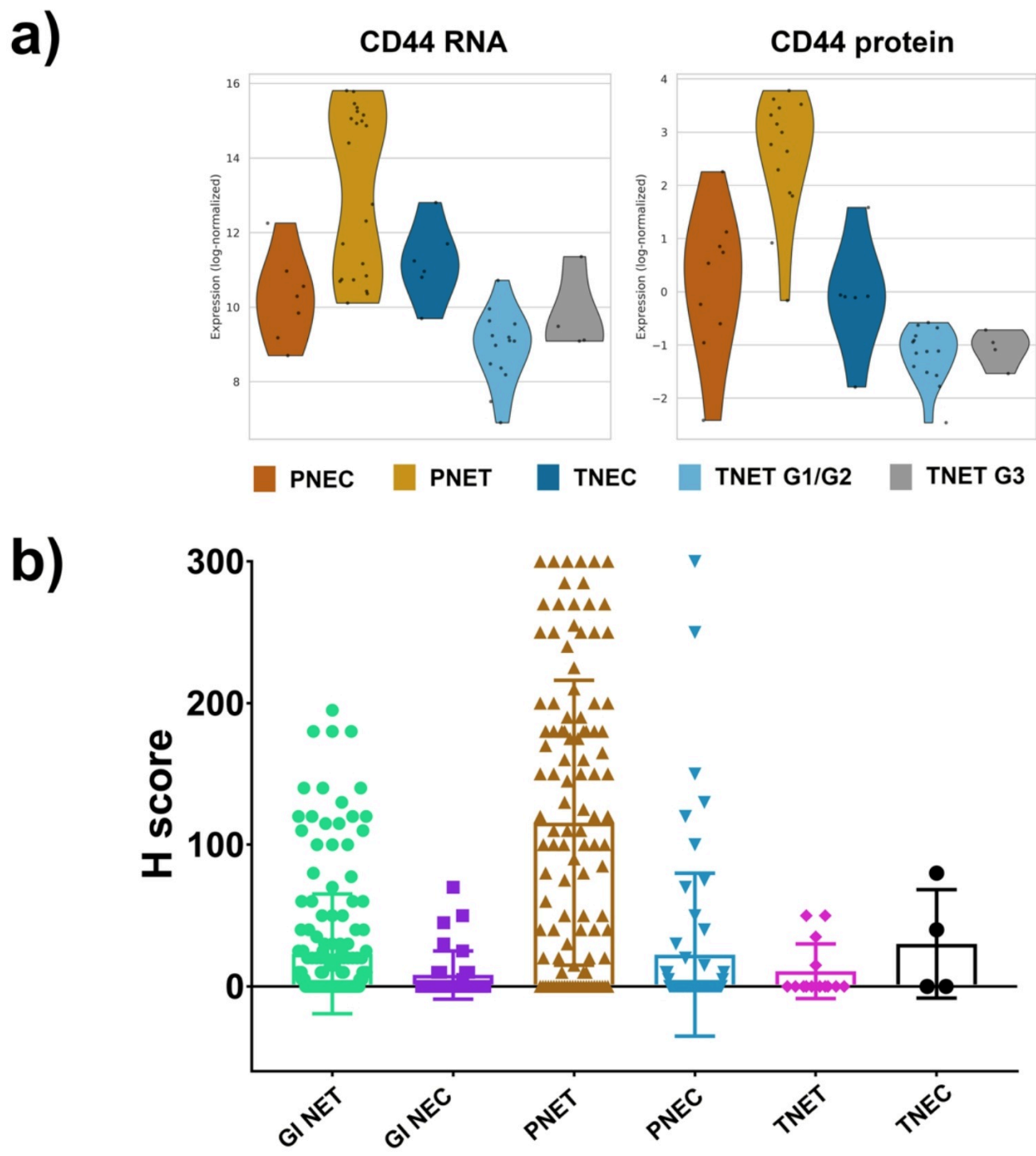


Figure 3



Figure 5

Integrated analysis of transcriptomic and proteomic data in different subgroups of thymic and pulmonary neuroendocrine neoplasms. NECs and NETs show opposing proliferative and metabolic programs. Pulmonary subtypes exhibit greater immune engagement, while thymic subtypes are characterized by EMT-driven invasion in TNEC and PI3K/AKT/mTOR-dependent growth in TNET.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1Variants.xlsx](#)
- [TNET2026OnlineResource.docx](#)